PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4 :
A61K 31/725, 31/73, C08B 37/08 A1

(11) International Publication Number: WO 86/05984

(43) International Publication Date: 23 October 1986 (23.10.86)

(21) International Application Number: PCT/SE86/00153

(22) International Filing Date: 2 April 1986 (02.04.86)

(31) Priority Application Number: 8501723-4

(32) Priority Date: 9 April 1985 (09.04.85)

(33) Priority Country: SE

(71) Applicant *(for all designated States except US)*: PHARMACIA AB [SE/SE]; S-751 82 Uppsala (SE).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : LINDBLAD, Gert, Tore, Östen [SE/SE]; Alvägen 22, S-752 45 Uppsala (SE).

(74) Agents: BERGANDER, Håkan et al.; Pharmacia AB, S-751 82 Uppsala (SE).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: HYALURONIC ACID PREPARATION TO BE USED FOR TREATING INFLAMMATIONS OF SKELETAL JOINTS

(57) Abstract

Hyaluronic acid preparation containing an effective amount of hyaluronic acid of a molecular weight exceeding 3 x 10^6 dalton, for intra-articular administration in the treatment of steroid arthropathy and progressive cartilage degeneration caused by proteoglycan degradation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GA	Gabon	MR	Mauritania
AU	Australia	GB	United Kingdom	MW	Malawi
BB	Barbados	HU	Hungary	NL	Netherlands
BE	Belgium	IT	Italy	NO	Norway
BG	Bulgaria	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TG	Togo
DK	Denmark	MC	Monaco	US	United States of America
FI	Finland	MG	Madagascar		
FR	France	ML	Mali		

"Hyaluronic acid preparation to be used for treating inflammations of skeletal joints".

This invention relates to high-molecular-weight hyaluronic acid having a molecular weight exceeding 3×10^6 dalton and intended for use as an agent for counteracting progressive cartilage destruction as, caused by degradation of proteoglycans. The type of treatment here referred to is intended for mammals, including man.

The term "hyaluronic acid", as employed here, refers both to the acid as such and to its physiologically acceptable salt, unless stated otherwise.

The quantitatively predominant part of articular cartilage consists of an extracellular matrix, which plays an important functional role and the composition of which is controlled by a relatively small number of cells. This matrix is composed of (i) collagen forming a fibrous network which is of importance for the volume stability of the tissue, and (ii), proteoglycans as a further major component, having a large amount of mutually repellent electric charges due to which the tissue acquires its elasticity and its ability to resist compression. Moreover, articular cartilage contains several other proteins, generally without known functions. An exception to this are the link proteins which participate in the formation of proteoglycan aggregates and contribute to the stability of these aggregates. Such aggregate formation is a necessary prerequisite for the fixation of the proteoglycans and its negatively charged groups in the tissue.

Degradation of the structures in articular cartilage is a typical characteristic of all diseases resulting in chronic destruction of the joint structures. Examples of such disorders are rheumatoid arthritis, psoriatic arthritis, and osteoarthrosis. Also, acute inflammation of a joint is often accompanied by destruction of the cartilage, although in most cases this will not develop into the chronically

destructive disease. It is not known which factors are crucial for the acutely inflamed joint to either proceed to healing or develop into the chronic process. Examples of diseases involving acute joint inflammation are yersinia arthritis, pyrophosphate arthritis, gout arthritis (arthritis urica), septic arthritis and various forms of arthritis of traumatic etiology. Among other factors potentially conducive to the destruction of articular cartilage may be mentioned, for instance, treatment with cortisone; this has been known for a long time to accelerate the degenerative process in osteoarthrosis. Such a so-called "steroid arthropathy" occurs far too often as an undersirable side effect of intra-articular cortisone treatment and can be avoided only by providing for a sufficiently long period of rest after the treatment. Steroid arthropathy is characterized by an advanced degree of articular destruction and X-ray-detectable changes of the same type as occur in advanced degenerative articular disease (Nizolek, DH & White, KK, Cornell Vet. 1981, 71:355-75). According to what is at present accepted as an explanation of the degenerative arthropathy development following treatment with cortisone, this arthropathy is believed to be caused by a primary effect on the chondrocyte metabolism. It should be noted, however, that the actual conditions prevailing in cases of arthritis with severe inflammation of the joint are of a rather more complex character, since in those cases injection of cortisone appears to have an overall positive effect on the clinical picture.

Hyaluronic acid is a naturally occurring glycosaminoglucan. Its molecular weight may vary from 50 000 dalton upwards, and it forms highly viscous solutions. As regards the actual molecular weight of hyaluronic acid in natural biological contexts, this is still a matter of much uncertainty: When the molecular weight of hyaluronic acid is to be determined, different values are obtained depending on the assay method employed, and on the source, the isolation method etc. Molecular weights given in this specification have been

determined according to Int J Biol Macr ol 7 (1985), p. 30-2. The acid occurs in animal tissue, e.g. spinal fluid, ocular fluid, synovial fluid, cockscombs, skin, and also in some streptococci. Various grades of hyaluronic acid have been obtained. It has been very difficult to obtain a sufficiently pure and high-molecular form suitable for administration in vivo. A preparation with a high degree of purity and entirely free from side effects is the non-inflammatory form described in US-A-4,141,973; this preparation is said to have a molecular weight exceeding 750,000 dalton, preferably exceeding 1,200,000 dalton and has been suggested for therapeutical use in various articular conditions for instance.

Heretofore, Applicant and others have carried out clinical studies on humans in respect of the indication "inflammatory joint conditions". Hyaluronate of a molecular weight substantially lower than 3×10^6 dalton (for example 10^6 dalton) was employed in these studies; it was applied by means of intra-articular injections. The accepted therapeutical method for the aforesaid indication is corticosteroid therapy, despite the fact that the effect of these substances is somewhat ambiguous, cf. above. Much work has been done also in the field of veterinary medicine, viz. in horses. Examples of scientific publications describing the use of hyaluronic acid for treatment of articular conditions are Nizolek, DK & White, KK (Cornell Vet. 1981, 71: 355-375); Namiki, O et al. (Int. J. Chem. Pharmacol., Therapy and Toxicol. 20 /1982/ p. 501-7); Asheim, A & Lindblad, G (Acta Vet. Scand. 17 /1976/ p. 379-94); Svanström, OG (Proceedings of the 24th annual convention of the American Association of Equine Practitioners; St Louis, Missouri, December 1978, Published 1978, p. 345-348); Wigren, A et al (Upsala J Med Sci Suppl 17 (1975) p. 1-20; and Gingerich, DA et al (Res Vet Sci 30 (1980) p. 192-97. In the patent literature hyaluronic acid preparations for general treatment of inflammatory conditions of the joints have been described. Examples are JP-A-58-37001, EP-A-138,572 (Mol. wt. within

certain ranges below 10^6 dalton), and EP-A-143,393 (hyaluronic acid having the following essential characteristics: free of nucleic acid and protein, derived from bacterial sources, and having a controlled Mol. wt. /2-4 x 10^6 dalton mentioned/). It should be noted that both EP-specifications were published during the priority year.

The positive effect of high-molecular-weight hyaluronic acid (>3 x 10^6 dalton) on progressive cartilage destruction involving proteoglycan degradation, has not been mentioned in the Prior Art.

The invention described in EP-A-145,681 has opened up new possibilities for studying early stages of cartilage destruction. The method measures how proteoglycans from cartilage are released into synovial fluid. An increased amount thereof in the synovial fluid is indicative of inceptive destruction, and thus the condition can be diagnosed long before any changes visible on X-ray or through the artroscope, have appeared in the joint. By means of this method it has been possible already at an early stage to monitor the effects exerted by various types of treatment upon the degeneration of cartilage. Our experiments now presented show that hyaluronic acid administered intra-articularly and having a molecular weight of about 3 x 10^6 dalton or more is prone to decrease the proteoglycan content of synovial fluid to almost normal levels. This indicates a positive effect on the proteoglycan metabolism of a joint. It has been shown that this is applicable both to inflammatory conditions and to degeneration caused by treatment with symptomatics, such as corticosteroid preparations. It is thus clear that a sufficiently high molecular weight of the hyaluronic acid is apt to counteract side effects that might be caused by corticosteroids or other symptomatics producing similar effects. This finding is quite unexpected, since when corticosteroids are applied, the amount of hyaluronic acid in the synovial cavity will increase substantially (see Experiment 1). Thus the hyaluronic acid employed has an

effect far exceeding that of the hyaluronic acid released due to the steroid treatment. Our experiments also show that these hyaluronic acid preparations have a very positive effect on such clinical symptoms as pain, swelling and lameness.

Objects to be attained by this invention are thus to provide improved therapeutical methods for, in the first place, early stages of cartilage degeneration, and to normalize proteoglycan metabolism, e.g. by preventing proteoglycan escape from the cartilage upon treatment with corticosteroids or other symptomatics having a similar effect.

These objectives are attained by intra-articular administration of an effective amount of hyaluronic acid with a mean molecular weight exceeding 3×10^6 dalton, preferably exceeding 4×10^6 dalton; but usually the molecular weight will not exceed 7×10^6 dalton. The dosage of hyaluronic acid administered should preferably be within the range of 5 mg - 80 mg. The amount of solution given at each administration is generally less than 60 ml, e.g. less than 20 ml, of an aqueous solution of the acid or its salt. It is convenient to administer the acid dissolved in water (< 2 % w/w, buffered to physiological pH), for instance in the form of a water-soluble sodium salt. The exact amount will depend on the particular joint to be treated. If the synovial cavity is large, the amounts required lie within the upper part of the aforesaid range, whereas, if the cavity is small, the amounts required are within the lower part of this range. In cases of severe and prolonged disorders, repeated administration may be necessary.

The therapeutic method of the invention may be carried out conjointly with a symptomatics treatment of a type as described above, involving a known administration of a therapeutically active amount of a corticosteroid or other symptomatics having a similar effect.

According to the invention, a hyaluronic acid is employed, which has been extracted from animal tissue known to contain hyaluronic acid of a molecular weight exceeding 3×10^6 dalton. Such hyaluronic acid may also be recovered from cell cultures producing it, e.g. by extraction. Sources that may be used are cockscombs (in the first place from White Leghorn) and certain bacteria, such as streptococci. The ability of a living organism to produce the "right" kind of hyaluronic acid is hereditary; consequently it is always necessary to carefully select individuals within the species employed.

The hyaluronic acid to be employed is heterologous, i.e. derived from a source other than the individual to be treated.

A suitable purification procedure is that described in the aforesaid patent US-A-4,141,973. Starting out from the right kind of source of raw material, it will be possible to obtain a substantially pure hyluronic acid having a mean molecular weight exceeding 3×10^6 dalton and having a protein content of less than 0.5 % (w/w).

The invention will now be further illustrated by means of a number of non-limitative examples. It will be seen very clearly from the examples that the effect exerted by the hyaluronic acid is very much dependent on the molecular weight of the hyaluronic acid administered. Generally speaking, a very low effect is obtained with molecular weights lower than 1.5×10^6 dalton, while, generally, a full inhibition of the proteoglycan release due to cartilage degeneration can be obtained in cases where the molecular weight exceeds 3×10^6 dalton.

EXPERIMENTALS

Clinical situation studied: Steroid arthropathy. The manner in which intra-articularly administered cortisone and hyaluronic acid of various molecular weights will affect the degeneration of cartilage has been studied by means of measuring synovial fluid levels of (i) proteoglycans (according to EP-A-145,681) and (ii) hyaluronic acid (according to Rowley, B. et al., Am. J. Vet. Res. 43 (1982), p. 1096-9).

Experiment 1

The animals treated were two race horses (trotters) which were no longer being used for racing but were exempt from clinical symptoms of articular problems. The following preparations were employed:

- Celestona®-Bifas® (liquid for injection, 6 mg of glucocorticoid per ml) (Schering Corp.)
- Na-hyaluronate dissolved in water (10 mg/ml, pH7.3, Mol. wt. = 3×10^6 dalton, produced according to US-A-4,141,973 from selected White Leghorn roosters /Pharmacia AB, Sweden/)
- Physiological saline.

8 joints of each horse were treated; doses given intra-articularly were the following:

Joint	Treatment
Fetlock L.F. (C1) U. carpal L.F. (C2)	4 ml of Celestona®-Bifas®
Fetlock R.F. (P1) U. carpal R.F. (P2)	6 ml of phys. saline

Fetlock L.L. (H1)	2 ml of Na-hyaluronate
M. carpal L.F. (R2)	
Fetlock R.L. (HC1)	4 ml of Celestona®-Bifas®
M. carpal R.F. (HC2)	2 ml of Na-hyaluronate

3 treatments were carried out: Day 0, after 1 week and after 2 weeks. Synovial fluid was sampled immediately before treatment on day 0, after 1 week and after 2 weeks; and 3 weeks, 4 weeks, 6 weeks and 8 weeks after start. Also, since it was desirable to measure the total amount of proteoglycan fragments and hyaluronic acid in the treated joints, the synovial fluid volumes were measured for this purpose by means of injecting isotope-labeled PVP and measuring the dilution. The results below thus give the total amounts of proteoglycans and Na-hyaluronate per joint.

Total amount of proteoglycans/joint (µg)

Horse No / Joint	L.1/I	L.1/II	P1/I	P1/II	H1/I	H2/II	HC1/I	HC1/II
Day 0	1 050	851	1 644	674	1 110	973	692	732
Week 1	22 838	15 661	1 123	865	442	061	14 374	2 417
" 2	20 744	16 772	936	680	484	423	13 574	1 061
" 3	19 404	13 501	1 549	579	399	390	8 731	1 089
" 4	6 193	926	872	477	455	456	1 085	428
" 6	1 086	441	643	204	-	246	253	316
" 8	693	383	623	293	230	260	227	318

Horse No	C2/I	C2/II	P2/I	P2/II	H2/I	H2/II	HC2/I	HC2/II
Day 0	1 012	890	1 033	1 022	1 067	992	830	606
Week 1	15 131	9 610	735	930	645	843	7 120	14 663
" 2	27 828	22 350	580	921	2 503	697	7 012	9 565
" 3	34 266	14 667	652	752	599	603	7 347	9 091
" 4	2 199	1 360	730	677	1 104	781	3 816	940
" 6	4 782	543	344	452	317	383	820	416
" 8	813	591	567	449	531	491	368	380

Total amount of Na-hyaluronate/joint (µg)

Joint Horse No	C1/I	C1/II	P1/I	P1/II	H1/I	H1/II	HC1/I	HC1/II
Day 0	4 079	4 081	6 500	5 673	5 939	9 800	-	10 481
Week 1	14 093	8 302	4 428	3 889	3 248	7 593	19 948	10 372
" 2	12 051	7 893	5 564	5 085	1 772	2 178	-	3 081
" 3	7 590	10 584	7 350	4 271	2 725	2 720	25 769	3 628
" 4	5 054	2 295	2 574	2 090	2 360	2 873	3 715	2 706
" 6	1 213	1 534	2 530	1 125	-	2 518	1 276	3 439
" 8	1 764	1 136	3 100	1 134	1 655	2 060	2 201	2 070

Horse No	C2/I	C2/II	P2/I	P2/II	H2/I	H2/II	HC2/I	HC2/II
Day 0	2 494	4 835	2 548	5 106	3 174	4 457	3 465	4 176
Week 1	7 900	9 091	3 007	5 472	3 978	5 414	9 900	14 935
" 2	14 901	11 858	3 050	4 644	5 218	2 425	14 760	13 377
" 3	16 102	12 178	3 776	5 264	4 450	3 599	25 359	5 244
" 4	3 323	6 588	3 338	3 575	4 477	3 348	8 848	3 820
" 6	4 329	4 236	2 070	3 474	1 800	2 796	3 686	3 042
" 8	5 264	3 588	3 617	2 460	3 876	3 186	4 080	2 256

The experiment shows that cortisone will break up the extracellular matrix in cartilage, thus explaining the known cartilage-degenerative effect of cortisone and/or its negative effect on chondrocytes. Hyaluronic acid having a molecular weight of 3 000 000 will reduce this effect and the duration thereof.

The results show also that cortisone gives rise to an increased amount of hyaluronic acid in the articular cavity.

The below Experiment 2 has been performed for elucidating whether or not the above effect on proteoglycan increase in the articular cavity was coupled to the molecular weight of the Na hyaluronate administered.

Experiment 2

3 horses (trotters) no longer actively engaged in races have been employed in this test: Horse M, Horse A and Horse H. Each horse was injected intraarticurlarly in the following joints (without any clinical symptoms of disease):

- Fetlock joint l, foreleg (A)
- Fetlock joint r, foreleg (B)
- Upper carpal l, foreleg (C)
- Upper carpal r, foreleg (D)

The above-enumerated joints of Horse M were treated with 4 ml of Celestona®-Bifas® (6 mg of cortisone/ml, Schering Corp.) together with 2 ml of Na hyaluronate having a molecular weight of about 10^5 dalton (1 % in water). The treatment was repeated at intervals of 1 week. Samples for measurement of proteoglycan fragment concentration in the synovial fluid were taken before the start of the treatment and 1 week after the second treatment.

The procedure followed in the case of Horse A was exactly the same except that the Na-hyaluronate had a molecular weight of 6×10^5 dalton. Also horse H was treated in exactly the same way, but with the exception that the Na-hyaluronate had a molecular weight of 3×10^6 dalton. Proteoglycan measurements were carried out in a manner analogous to Experiment 1. Results of the analyses (mg/l) are set forth in the Table:

Time	Joint	Horse M (100 000)		Horse A (600 000)		Horse H (2 700 000)	
0	A	34,4		20,3		32,5	
	B	45,9		21,1		27,9	
	C	33,6	$\bar{x}$=37,2	17,6	$\bar{x}$=21,3	26,6	$\bar{x}$=28,0
	D	35,1		26,1		24,8	
after 1 week	A	534,6		374,1		311,4	
	B	550,8		18,1		136,4	
	C	858,6	$\bar{x}$=494,9	101,5	$\bar{x}$=260,5	46,0	$\bar{x}$=130,5
	D	35,3		548,1		28,1	
after 2 weeks	A	350,5		169,4		54,4	
	B	615,6		23,8		43,0	
	C	118,9	$\bar{x}$=280,6	20,4	$\bar{x}$=62,7	116,1	$\bar{x}$=58,0
	D	37,4		37,0		18,5	

The results show that the proteoglycan release (the degeneration) decreases with increasing molecular weights of Na-hyaluronate. When the molecular weights rise above 3×10^6, the proteoglycan release is hardly measurable at all with the method employed.

Experiment 3

Two horses, no longer in active training or racing, were used. The actual joints: both fore-fetlock joints in both horses (1 and 2) and both middle carpal divisions in one of them (3), were considered free from clinical signs of arthritis.

After a synovial fluid sample had been withdrawn, 0.5 ml of a 1 % Carrageenan solution was injected into each of the abovementioned joints. After 8 hours, the joints were emptied of the synovial fluid and treated: right joints with 2 ml 1 % sodium hyaluronate solution (molecular weight 4 x 10^6 dalton) and left joints with the same amount of saline. Synovial fluid samples were then withdrawn at the following intervals: 24, 48, 96, 120 and 168 hours.

All joints reacted with a severe synovitis, typified by lameness, swelling, pain and heat. The results of the proteoglycan determinations are shown in Fig. 1.

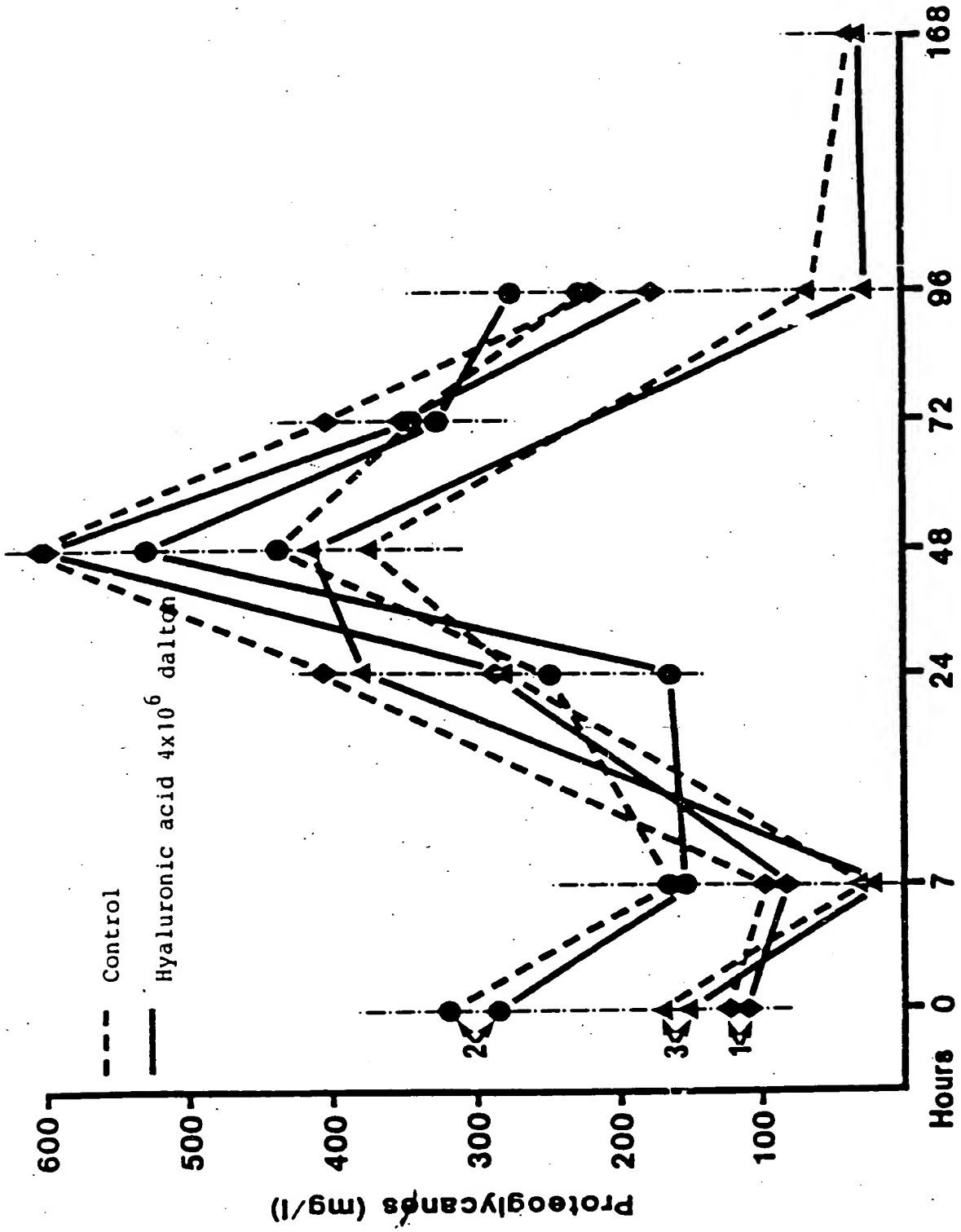
The hyaluronate-treated joints showed a remarkable clinical improvement immediately after the treatment and could be considered clinically sound within 2 days. The NaCl-treated joints remainded sore with light to moderate signs of swelling, pain and lameness the last follow-up day (7 days after the injection). The results obtained are very interesting and can be interpreted as carrageenan eliciting a proteoglycan-degrading effect together with a disturbing effect on the removal of proteoglycans from the synovial fluid.

The invention is characterized in more detail in the attached claims forming an integral part of this specification.

C L A I M S

1. A hyaluronic acid preparation containing an effective amount of hyaluronic acid of a molecular weight exceeding 3×10^6 dalton, for intraarticular administration in the treatment of progressive cartilage degeneration caused by proteoglycan degradation.

2. A hyaluronic acid preparation containing an effective amount of hyaluronic acid of a molecular weight exceeding 3×10^6 dalton, for intra-articular administration for the purpose of preventing steroid arthropathy.


Proteoglycans (mg/l)
600
500
400
300
200
100
Hours
0
7
24
48
72
96
168
Control
Hyaluronic acid $4x10^6$ dalton

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE86/00153

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC 4

A 61 K 31.725, 31/73, C 08 B 37/03

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
IPC 2.3.4	A 61 K 31/725, /73, /735; C 08 B 37/08
IPC 1	A 61 k 27/00
US Cl	424:180; 514:23, 54

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched *

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	US, A, 4 141 973 (BIOTRICS, INC) 27 February 1979 See inter alia claims 1-4 and column 14, lines 7-48	1-2
X	Upsala Journal of Medical Sciences, Supplement 17, issued 1975 (Uppsala), A. Wigren et al, "Repeated intraarticular implantation of hyaluronic acid", see pages 1-20, especially page 3 and page 19 ("Summary")	1-2
X	Proceedings of the twenty-fourth annual convention of the American Association of Equine Practitioners, St. Louis, Missouri, 2-6 December 1978, published 1979 by American Association of Equine Practitioners, O.G. Swanström, "Hyaluronate (hyaluronic acid) and its use", see pages 345-348, especially p. 345, first two paragraphs, and p. 347, second paragraph.	1-2
X	Research in Veterinary Science, Volume 30, No. 2, issued 1981 (Oxford), D.A. Gingerich et al, "Effect of exogenous hyaluronic acid	1-2

* Special categories of cited documents: **

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1986-06-30	1986 -07- 04
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Martin Hjälmdahl Martin Hjälmdahl

L.E

Form PCT/ISA/210 (second sheet) (January 1985)

III DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	on joint function in experimentally induced equine osteoarthritis: dosage titration studies", see pages 192-197, especially p. 192 and p. 196-197 ("Discussion")	
X	International Journal of Clinical Pharmacology, Therapy and Toxicology, Volume 20, No. 11, issued 1982 (München), O. Namiki et al, "Therapeutic effect of intra-articular injection of high molecular weight hyaluronic acid on osteoarthritis of the knee", see pages 501-507, especially p. 501 and p. 504-506 ("Discussion".	1-2
X	Patent Abstracts of Japan, Vol. 7, No. 118, C-167, abstract of JP 58-37001 (A), published 1983-03-04	1-2
P	EP, A2, 0 138 572 (FIDIA SPA) 24 April 1985 See inter alia claims 18-20, page 19, second paragraph, and pages 22-23 (Examples 13-15)	1-2
P	EP, A2, 0 143 393 (MILES LABORATORIES, INC) 5 June 1985 See inter alia the claims, page 16 (table V) and page 21, line 6- page 23, line 8.	1-2